STUDY ABOUT THE LEVELS OF SERUM GLYCOPROTEINS IN PATIENTS WITH DEPRESSIVE DISORDER

Dissertation Submitted to

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BRANCH – XIII

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI, INDIA.

MARCH 2007

CERTIFICATE

This is to certify that the dissertation entitled "**STUDY ABOUT THE LEVELS OF SERUM GLYCOPROTEINS IN PATIENTS WITH DEPRESSIVE DISORDER"** is the bonafide original work of **Dr. N. DHEEBALAKSHMI** in partial fulfillment of the requirements for **M.D. (BIOCHEMISTRY) BRANCH – XIII** Examination of the Tamilnadu Dr. M.G.R. Medical University to be held in March 2007.

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DECLARATION

I, **Dr. N. DHEEBALAKSHMI,** solemnly declare that dissertation titled, "**STUDY ABOUT THE LEVELS OF SERUM GLYCOPROTEINS IN PATIENTS WITH DEPRESSIVE DISORDER"** is a bonafide work done by me at Govt. Stanley Medical College & Hospital during 2004-2007 under the guidance and supervision of **Dr. P. JAYANTHI, M.D.** Professor and Head, Department of Biochemistry, Stanley Medical College, Chennai-600 001.

The dissertation is submitted to Tamilnadu, Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (BRANCH – XIII) in Biochemistry.**

Place : Chennai.

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CONTENTS

ABBREVIATIONS USED

1)	UDP – Gal	-	Uridine Diphosphate – galactose
2)	UDP – Glc	-	Uridine diphosphate – glucose
3)	GDP – Man	-	Guanosine diphosphate – Mannose
4)	CMP – Neu	-	Cytidine Monophosphate – N- Acetyl neuraminic acid.
5)	GDP – Fuc	-	Guanosine diphosphate – fucose
6)	UDP – Gal NAc	-	Uridine diphosphate N- Acetyl galactosamine
7)	UDP – Glc NAc	-	Uridine Diphosphate N acetyl glucosamine
8)	UDP – Xyl	-	Uridine diphosphate – Xylose
9)	Gal	-	Galactose
10)	Glc	-	Glucose
11)	Man	-	Mannose
12)	GPI	-	Glycosyl phosphatidyl Inositol.
13)	Glc NAc–Ser(thr)	-	N – acetyl Glucosamine – Serine (Threonine)
14)	Asn – Glc NAc	-	Asparagine – N – Acetyl glucosamine
15)	ZPI – 3	-	Zonapellucida Glycoprotein 1-3
16)	CD 34	-	Cluster designation – 34
17)	Gly CAM 1	-	Glycan bearing cell adhesion molecule 1
18)	Mac–1	-	Macrophage associated Antigen – 1
19)	ICAM	-	Intercellular adhesion Molecule
20)	LFA	-	Lymphocyte function associated antigen
21)	PECAM	-	Platelet Endothelial cell adhesion Molecule
22)	ER	-	Endoplasmic Reticulum
23)	DST	-	Dexamethasone Challenge test
24)	ICD – 10	-	International Statistical Classification of diseases and related health problems
25)	WHO	-	World Health Organisation
26)	DSM IV	-	Diagnostic and statistical manual of mental disorders
27)	OD	-	Optical density

28) SD - Standard Deviation

29) PBH - Protein Bound Hexose

30) PBHx - Protein Bound Hexosamine

31) NaoH - Sodium Hydroxide

32) Hcl - Hydrochloric Acid

33) H_2SO_4 - Sulphuric acid

34) Na_2CO_3 - Sodium Carbonate

ACKNOWLEDGEMENT

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I express many thanks to all the technical staff and other staff of the department of Biochemistry, Stanley Medical College for their kind cooperation to carry out this work successfully.

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BIBLIOGRAPHY

1) Winzler R.J. Advances in Cancer Research Vo.1. Academic Press, Newyork , 1953. p 503.

2) Winzler R.J. (1955). Determination of serum glycoproteins. Methods of biochemical analysis. Vo.2. Ed. Glick page 299-311, Interscience Publishers, Newyork, USA.

3) U. Singh, R.L. Solanki, V.K. Anand and I.N. Ramdeo (1989) A Study of Serum Glycoprotein in Pulmonary tuberculosis.

4) Dutt, M. Chatterjee, A.K. Khanade J.M. and Rajan S.R. (1975). Serum Glycoproteins in patients of Ischemic Heart Disease and Normal Controls. Indian J. Med. Res. 63, 282 – 285.

5) Bhuvarahamurthy, V. Balasubramanian, N. Vijayakumar S. and Govindswamy S (1995) : Effect of radiation on serum glycoproteins and glycosidases in patients with cervical carcinoma. Int. J. Gynaecol. Obstet, 48, 49-54.

6) Varma, R. and Hoshino. A.Y. (1980) Serum glycoprotein in Schizophrenia. Carbohydr. Res. 82, 343-351.

7) Mukesh Nandav, S.K. Ojha, Ranjit Kaur. Changes in levels of serum glycoproteins in major depressive disorders. Indian Journal of clinical biochemistry, 2005, 20(2) 154-157.

8) Opeolu M. Adeoye, Robert E. Ferrell, Margaret A.Krishner, Benoit H. Mulsant, Karen Seligman, Amy E. Begley, Charles F. Reynolds III, and Bruce G. Pollock. Journal of Geriatric Psychiatry and Neurology. Vol.16, No.4 Dec. 2003.

9) Nieto E, Vieta E, Alvaroz L, et al. Alpha 1 – acid glycoprotein in Major depressive disorder. Relationship to severity, response to treatment and imipramine plasma levels. J. affect disord 2000; 59 : 159 – 164.

10) Narendra G. Mehta and Alamela Ventakaraman (1973). Serum Glycoproteins levels and disease – an evaluation. Indian J Med. Res. 63, 970 – 976.

11) Fatemi SH, Kroll JL, Stary J.M. Altered levels of Reelin and its isoforms in mood disorders and schizophrenia. Neuroreport 2001 Oct. 29; 12(15) : 3209 – 15.

12) Sluzewska A, Rybakowski JK, Sobieska M, Wiktorowicz K. Concentration and Microheterogenicity glycophorms of alpha – 1-acid glycoprotein in Major depressive disorder. J. Affect Disord. 1996 Jul. 8 ; 39(2) ; 149-55.

13) Bondy B, Baghai TC, Minov C, Schule C, Schwarz MJ et al. Substance P serum levels are inceased in Major depression. Biol Psychiatry, 2003 Mar. 15 ; 53 (6) : 538 – 42.

14) Healy D, Calvin J, White house AM et al. Alpha-1- acid glycoprotein in major depressive and eating disorders. J. Affect disord. 1991; 22 : 13-20.

15) Kehoe WA, Kwentus JA, Sheffel WB, Harralson AF, Increased alpha-1-acid glycoprotein in depression lowers free fraction of imipramine. Biol Psychiatry 1991; 29: 489 – 493.

16) Bruce EC, Mussel Man DL. Depression, alterations in platelet function and Ischemic heart disease. Psychosom Med. 2005; 67 (Suppl 1) : S34 – 6.

17) Robert K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rod Well. Harper's Illustrated Biochemistry. 26th Edition Page 514 – 534. Mc. Graw Hill.

18) Schmid K.J. Am. Chem. Soc. 75, 2532 (1953).

19) Robert K. Murrey, Daryl K. Granner, Peter A : Mayer Victor W. Rodwell. Harper's Biochemistry 25th Edition Page 675 – 694 Mc. Graw Hill.

20) Werner, I. Acta Physiol. Scand 19, 27 (1949).

21) Jeremy M. Berg, John L. Tymoczko, Lubert Stryer, Biochemistry. 5th Edition. W.H. Feeman and company page 306 – 310.

22) M.G. Golder, Juan J. Lopoz – Ibor Jr. Nancy C. Andreasen. New oxford Text book of psychiatry. Vo.1, page 677-736. Oxford University press Inc. New york. Reprinted 2004. First published 2000.

23) Benjamin J. Sadoc, Virginia A. Sadock. Kaplan & Sadock . Comprehensive Text Book of Psychiatry. 7th Edition Vol. 2, Page 2740 – 2757. Lippincott Willians and Wilins.

24) The ICD – 10 Classification of Mental and Behavioural disorders – clinical descriptions and diagnostic guidelines. World health organization. Geneva. Page 81-87. A.I.T.B.S. Publishers and Distributors.

25) Gillespie CF, Nemoroff CB. Hypercortisolemia and depression. Psychosom Med. 2005; 67 (Suppl.1) S 26-8.

26) Shetlar M.R, Foster J.V. Koelly, K.H. Shetlar, C.L. Bryan. R.S. and Everett M.R. Cancer Research 9, 515 (1949).

27) Shetlar M.R., Kelly, K.H. Foster, J.V. shetlar, C.L. and Everdt. M.R. Am. J. Obstet. Gynecol, 59, 1140.

28) Fran Van Hunsel, Annick Waulers, Eric Vandoolaeghe et al. low total serum proteins, albumin and Beta and gamma – globulin in major and treatment – resistant depression. Effects of antidepressant treatments. Psychiatry Research Vol. 65, Issue 3, 1996 Page 159 – 169.

29) Stanley L. Robbins, Ramzi S, Cotran Kumar et al. pathologic basis of diseases. 7th Edition. Saunders Page 65.

30) Nemer off CB, Krishnan KR, Blazer DG, Knight DL, Benjamin D, Meyerson LR. Elevated plasma concentrations of α – 1 acid glycoprotein, a putative endogenous inhibitor of the tritiated imipramin binding site, in depressed patients. Arch Gen Psychiatry, 1990 Apr. 47 (4), 337-40.

31) Schuld A, Schmid DA, Haack M, Holsboer F, Friess E, Pollmacher T. Hypothalamo – pituitary – adrenal function in patients with depressive disorders is correlated with baseline cytokines levels, but not with cytokine response to hydrocortisone. J Psychiatr Res 2003, Nov – Dec 37(6); 463 – 70.

32) Seidel A, Arolt V, Hunstiger M, Renk L, Behnisch A, Kirchner H. Cytokine production and serum proteins in depression. Scand J. Immunol – 1995 Jun. 41(6) 534 – 8.

33) Maes M, Scharpe S, Cosyns P, Heltzer H. Relationships between basal hypothalamic pituitary – thyroid axis activity and plasma haptoglobin levels in depression. J Psychiatr Res, 1994 Mar – Apr 28(2); 123 – 34.

34) Song C, Dinan T, Leonard BE. Changes in immunoglobulin, Complement and acute phase protein levels in the depressed patients and normal controls. J. Affect Disord 1994; Apr 30(4); 283-8.

35) Maes M, Scharpe S, Meltzer HY, Bosmans E, Suy E, Calabrese J, Cosyns P. Relationship between Interleukin – 6 activity, acutephase proteins and function of the hypothalamic – pituitary – adrenal axis in severe depression. Psychiatry Res 1993 Oct. 49(1) 11-27.

36) Joyce PR, Hawes CR, Mulder RT, Seliman JD, Wilson DA, Bosewell DR. Elevated levels of acute phase plasma proteins in major depression. Biol psychiatry 1992, Dec 1 32 (11). 1035 -41.

37) Maes M, Scharpe S, Van Grotel L, Uyttenbroeck W, Cooreman W, Cosyns P. Suy E. Higher alpha 1 antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression - further evidence for the existence of an inflammatory response during that illness. J Affect Disor 1992, Mar ; 24(3). 183-192.

38) Schlechte JA, Coffman T. Plasma free cortisol in depressive illness – a review of findings and clinical implications. Psychiatr Med. 1985; 3(1) 23 to 31.

39) Hornig M, Goodman DB, Kamoun M, Amsterdam JD. Positive and negative acute phase proteins in affective subtypes. J Affect disord 1998 Apr. 49(1); 9-18.

INTRODUCTION

The presence of protein bound carbohydrate in serum has been recognized for many years. The early work in this field was reviewed in 1929 by Grevenstuk in an extensive paper and also in 1933 by Rimington.

Many investigators have demonstrated that the concentration of the glycoprotein in human serum is abnormally high in a number of physiological and pathological states[1]. Evidence has accumulated showing that there are a number of distinct serum glycoproteins and that the concentrations of these may vary in an independent manner[2].

Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion. Glycoproteins as a group have multiple and complex function and are found as enzymes, hormones, blood group substances and as constituents of extra cellular membrane. These are organic compounds composed of both a protein and carbohydrates usually hexose, hexosamine, fucose, sialic acid joined together covalently linked to polypeptide chain. The level of different types of serum glycoproteins are maintained within a narrow range in health but is elevated in many pathological conditions like tuberculosis[3], autoimmune disease,

cardiovascular disease, Ischemic heart disease[4], Diabetes Mellitus, cancer cervix[5], uterus and breast, psychiatric disorder[2].

In psychiatric disorder such as schizophrenia, elevated levels of serum glycoprotein has been reported[6]. The present study is to evaluate the changes in the level of serum glycoproteins as protein bound hexose, protein bound hexosamine in patients with depressive disorder and compared with normal healthy subjects as control. Most of the work has involved determination of total glycoproteins from the content of protein bound hexose and protein bound hexosamine[2].

AIM AND OBJECTIVES

In psychiatric diseases, Major depressive disorder is most common. The glycoproteins vary in their relative content of serum of four monsaccharides. The net change in the total level would also vary depending upon the monosaccharide used for determination. In psychiatric disorders such as schizophrenia, elevated levels of serum glycoprotein has been reported.

It was thought of interest to investigate the changes in the levels of serum glycoproteins in patients with depressive disorder.

The aim of this study is to estimate and compare the level of serum glycoproteins as protein bound hexose and protein bound hexosamine in patients with severe depressive disorder and normal subjects.

To evaluate whether the levels of serum glycoproteins are elevated in depressive disorder and serve as a marker in diagnosis of the patients with severe depression.

REVIEW OF LITERATURE

A great number of studies have indicated that the concentration of the serum glycoproteins may be makedly increased in patients or in experimental animals suffering from a wide variety of pathological conditions.

Most of the work in this field has involved determination of the total Glycoproteins from the content of protein bound hexose or hexosamine.

Mukesh Nandave, SK Ojha et al reported the changes in levels of serum glycoproteins in Major depressive disorder. He had concluded that serum glycoprotein levels may serve as an indicator of Major depressive disorders[7].

Opeolu M. Adoeye, Robert E. Ferrell et al reported that Alpha 1 acid glycoprotein level rise as a result of increased hepatic synthesis stimulated by the inflammatory cytokines[8].

Nieto E, vieta E Alvarez et al reported that plasma level of alpha 1 Acid glycoprotein is increased in Major depressive disorder and its relationship to severity, response to treatment and imipramine plasma levels[9].

Narendra G. Mehta and Alamela Venkataraman had studied the level of serum glycoprotein in various diseases[10].

Fatemi SH, Kroll JL, Stary JK[11] had studied the altered levels of Reelin and its isoforms in mood disorders (Bipolar disorder and major depression) and schizophrenia. They reported that blood Reelin levels and its isoforms may be used as potential peripheral markers to diagnose presence of several psychiatric disorders and may also serve as targets for further therapeutic interventions.

Sluzewska A, Rybakowski JK et al[12] reported high level of Alpha -1-acid glycoproteins and its microheterogencity in patients with major depressive disorder.

Bondy B. Baghai TC, Minov C et al[13] reported increased levels of substance 'P' in major depression.

Healy D, Calvin J, White house AM et al[14] studied about the level of Alpha -1- acid glycoprotein in major depressive and eating disorders.

Kehoe WA, Kwentus JA, Sheffol WB, Harralson AF[15], reported increased alpha 1 – acid glycoprotein in depression lowers free fraction of imipramine.

Bruce EC, Mussel Man DL[16] studied about depression, alterations in platelet function and Ischemic heart disease. They observed that depression is also associated with complex platelet abnormalities.

GLYCOPROTEINS

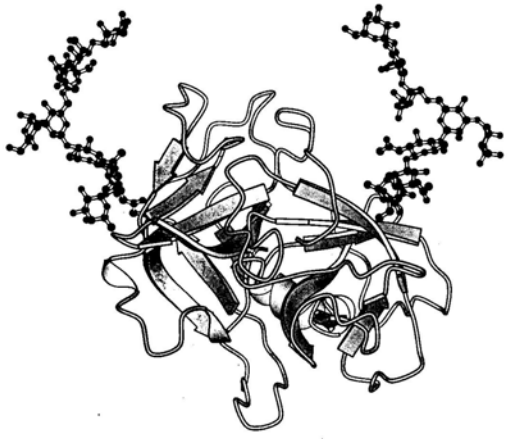
TERMINOLOGY[2]

The committee on protein Nomenclature of the American Physiological society and the American Society of Biochemists defined Glycoproteins as, “the compounds of the protein molecules with a substance or substances containing a carbohydrate group other than Nucleic acids”.

The amount of protein bound carbohydrate in serum is a direct measure of the serum glycoprotein levels, as carbohydrates other than glucose is a constituent of many serum proteins i.e. glycoproteins.

The serum glycoprotein levels were most frequently been determined and expressed in terms of hexose and hexosamine content.

GLYCOPROTEIN SHOWING LINKED CARBOHYDRATES ON ITS SURFACE.



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TYPES OF GLYCOPROTEINS

Glycoproteins may be two general types[2].

They are

1) Mucoproteins
2) Mucoids.

    1) Mucoproteins are proteins bonded to carbohydrate through polar linkage which are relatively easily split in an electric field, by concentrated salt or by alkaline solutions.

       Eg : - Mucopoly saccharides

    2) Mucoids are glycoproteins in which the carbohydrate is covalently bonded to the protein.

       Eg : - ovo mucoid, seromucoid

The extensively studied serum “Mucoprotein” of winzler et al is considered as having a mucoid nature since the carbohydrates can be split from the protein by drastic treatment. This is a heterogenous fraction that is left in solution by perchloric acid and precipitated by phosphotungstic acid. It is essentially identical with the serum fraction isolated in 1897 by Zanotti et al from filtrates of serum deproteinated by heat coagulation and subsequently studied Bywaters and Rimington.

The fraction was designated as seromucoid by all of the early investigators. It is appropriate to replace the term plasma mucoprotein with the established term seromucoid.

The Major component in human seromucoid is an electophoretically and ultracentrifugally distinct acidic glycoprotein which has been crystallized and quite well characterized by chemical, physical and immunological Method.

NATURE OF SERUM GLYCOPROTEINS[17]

About 200 Monosaccharides are found in Nature. However only eight are commonly found in oligosaccharide chains of glycoproteins.

The principal sugars found in human glycoproteins are as follows.

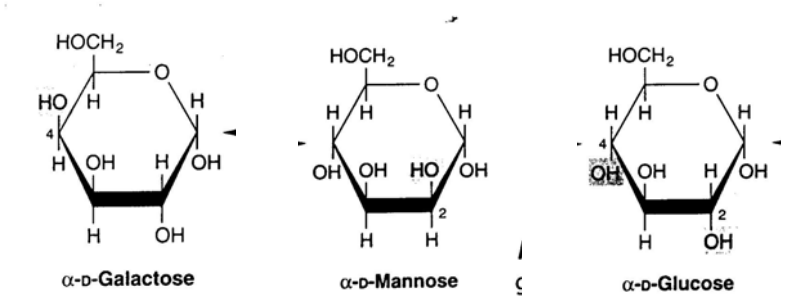
S.No	Sugar	Type	Abbreviation	Nucleotide Sugar
1.	Galactose	Hexose	Gal	UDP-Gal
2.	Glucose	Hexose	Glc	UDP-Glc
3.	Mannose	Hexose	Man	GDP – Man
4.	N-Acetyl neuraminic acid	Sialic acid	Neu Ac	CMP – Neu Ac

5.	Fucose	Deoxy hexose	Fuc	GDP – Fuc
6.	N-Acetyl galactosamine	Amino hexose	Gal NAc	UDP – Gal NAc
7.	N-Acetyl glucosamine	Amino hexoxe	GlC NAc	UDP – Glc NAc
8.	Xylose	Pentose	Xyl	UDP - xyl

Mannose is the common sugar in N-linked glycoproteins. N-Acetyl neuraminic Acid is usually found at the terminals of oligosaccharide chains, attached to sub terminal galactose or N-acetyl galactosamine residues.

The other sugars listed are generally found in more internal positions. Sulfate is often found in glycoproteins usually attached to Gal, Gal NAc or Glc NAc.

Structure of principal sugars found in Human glycoproteins



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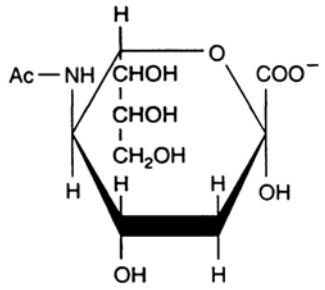
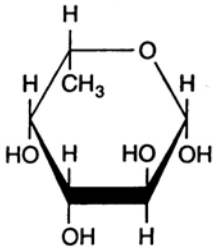
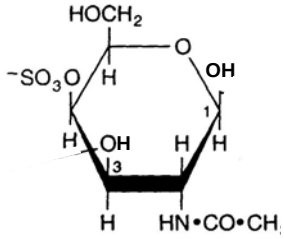
N- ACETYL NEURAMINIC ACID



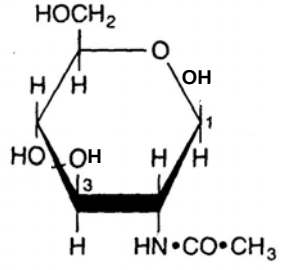
β – L FUCOSE (6 – DEOXY β – L – GALACTOSE)



N- ACETYLGLALACTOSAMINE SULFATE



N-ACETYLGLUCOSAMINE



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On the average the normal human serum contains[2],

1) Protein bound hexose 121 $\pm$ 2.1 mg% as galactose – Mannose

2) Protein bound hexosamine 83.4 $\pm$ 4.1 mg%

3) Protein bound fucose 8.9 $\pm$ 0.6 mg%

4) Protein sialic acid 60 $\pm$ 3.1 mg%

The galactose and Mannose are present in about equal amounts.

The protein bound carbohydrates of serum distributed among different protein fractions. The partition of serum into fractions of varying carbohydrates content has been carried out by neutral salts, ethanol precipitation and by electrophoresis.

Orosomucoid, the acidic glycoprotein isolated from human serum by Schmid.K. and by Weimer et al has been shown to have the mobility of α_1 globulin in veronal buffer at pH 8.6.

Schmid.K[18] has demonstrated in the supernatant from fraction V prepared by low temperature – low salt – ethanol method, three additional low molecular weight, acidic glycoproteins with mobilities of α_2 globulin at pH8.6. The hexose and hexosamine contents of these glycoproteins were between 6 & 4%.

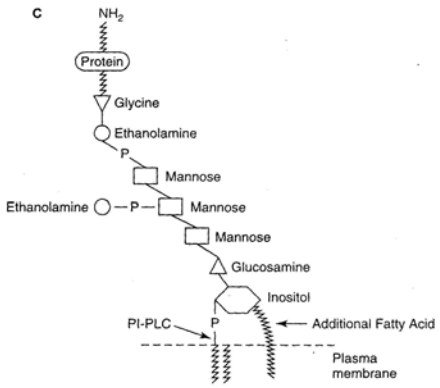
The other carbohydrate containing proteins that have been demonstrated in serum include prothrombin, gonadotropin, c_4 component of complement.

MAJOR CLASSES OF GLYCOPROTEINS[19]

Glycoproteins are divided into 3 major classes based on the nature of linkage between their polypeptide chains and the oligosaccharide chains.

1) Those containing an O-glycosidic linkage (o-linked) involving the hydroxyl side chain of serine or threonine and a sugar such as N-acetyl galactosamine.
2) Those containing N – glycosidic linkage (N-linked) involving the amide nitrogen of asparagine and N-acetyl glucosamine.
3) Those linked to the carboxyl terminal amino acid of a protein via a phosphoryl- ethanolamine moiety joined to an oligosaccharide which in turn is linked via glucosamine to phosphatidyl inositol. This is referred as GPI – anchored (Glycosyl phosphatidyl Inositol. Anchored) Glycoproteins
4) Glycophorin-an important red cell membrane glycoprotein contains both o- and N- linked oligosaccharides.

Glycosyl Phosphatidyl Inositol Linkage



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SUBCLASSES OF O-GLYCOSIDIC LINKAGES

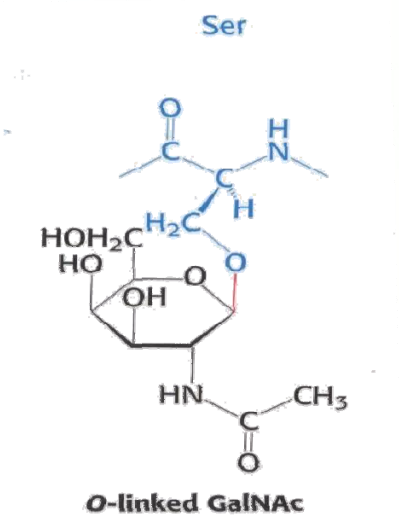
1) Gal NAc – Ser (Thr) linkage is the predominant linkage.

   Two typical oligosaccharide chains found in member of this subclass are sub maxillary mucins and Fetuin and in the sialoglycoprotein of the membrane of human red blood cells.

2) Proteoglycans contain a Gal – Gal – xyl – Ser trisaccharide. (link trisaccharide)

3) Collagens contain a Gal – hydroxy lysine linkage.

4) Nuclear proteins and cytosolic proteins contain side chains consiting of a single GlcNAc attached to a serine or threonine residues [GluNAc – Ser (Thr)]



O-linked GalNAc

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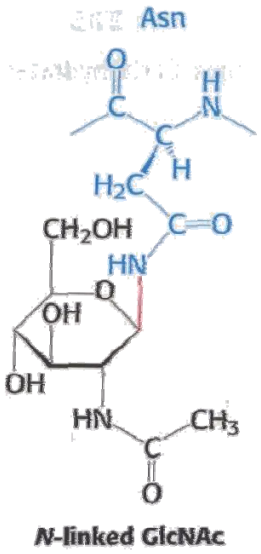
CLASSES OF N-TERMINAL LINKED GLY COPROTEINS

- The three major classes of N-linked oligosaccharides are complex, hybrid and high – mannose.
- N-linked glycoprotein is the major class of glycoprotein.
- They are distinguished by the presence of their Asn – GlcNAc linkage.
- It includes both membrane bound and circulating glycoproteins.
- Each type share a common pentasacchacide, Man3GlcNac2.
- The presence of the common pentasaccharide is explained by the fact that all three classes share an initial common mechanism of Biosynthesis.
- Glycoproteins of complex type generally contains terminal NeuAc residues and underlying Gal and GlcNAc residues, the latter often constituting the disaccharide N-acetyl lactosamine.
- Repeating N-acetyl actosamine units are often found on N- linked glycan chains.
- The oligosaccharide branches are often referred to as antennae, so that bi, tri and tera and penta – antennary structures may all the found.
- High – mannose oligosaccharides typically have two to six additional Man residues linked to the pentasaccharide core.

- Hybrid molecules contain features of both of the two other classes



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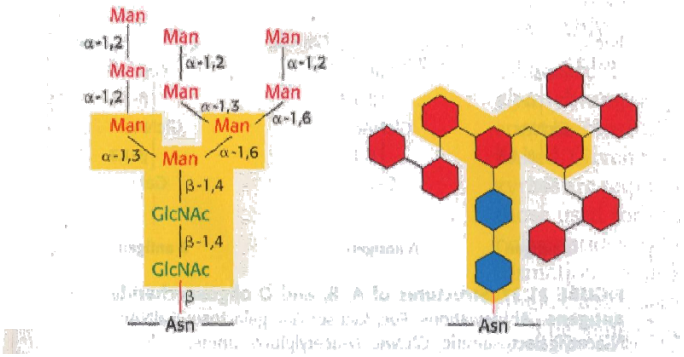
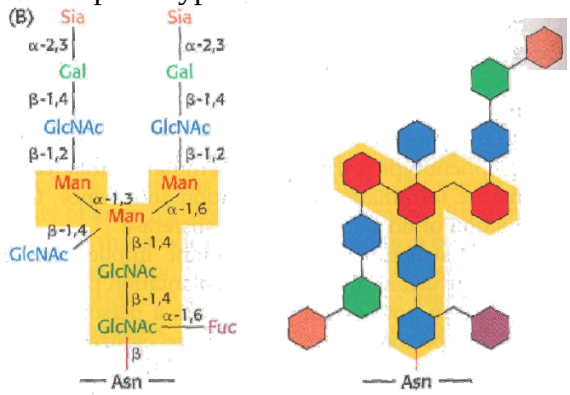
(A) N Linked Oligosaccharides – High Mannose Type



Complex Type



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FUNCTIONS OF GLYCOPROTEINS:

The oligosaccharide chains encode considerable biological information and this depends on upon their constituent sugars, their sequences and their linkages.

The functions of **oligosaccharide chains** of glycoprotein are.

1) Modulate physico chemical properties.

   Eg:- Solubility, viscosity, charge, conformation, denaturation, binding sites for bacteria and virus.

2) Protect against proteolysis, from inside and outside of the cell.
3) Affect proteolytic processing of precursor proteins to smaller products.
4) Involved in biological activity. Eg. Humanchorionic gonadotropin.
5) Affect insertion into membranes, intracellular migration, sorting and secretion.
6) Affect embryonic development and differentiation.
7) May affect sites of metastases selected by cancer cells.

FUNCTIONS SERVED BY GLYCOPROTEINS

S.No.	Function	Glycoprotein
1)	Structural Molecule	Collagens
2)	Lubricant and protective agent	Mucins
3)	Transport Molecule	Transferrin ceruloplasmin
4)	Immunologic molecule	Immunoglobulins, histocompatibility antigens.
5)	Hormone	Choronic gonadotropin, thyroid stimulating hormone.
6)	Enzyme	Various eg. Alkaline phosphatase
7)	Cell attachment – recognition site	Various proteins involved in cell-cell, virus – cell, bacterium-cell, and hormone – cell interactions.

8) Antifreeze	Certain plasma proteins of cold water fish.
9) Interact with specific carbohydrates	Lectins, selectins, antibodies
10) Receptor	Various proteins involved in hormone and drug action
11) Affect folding of certain proteins	Calnexin, calreticulin.

ROLE OF GLYCOPROTEIN IN FERTILIZATION:-

- Zona pellucida (ZP), a thick transparent, non cellular envelope surrounds the oocyte.
- It has three glycoprotein, ZP 1-3. Of particular note is ZP3, an O-linked glycoprotein that function as a receptor for the sperm.
- A protein on the sperm surface, possibly galactosyl transferase, interacts specifically with oligosaccharide chains of ZP3.
- This interaction by transmembrane signaling, induces the acrosomal reaction, in which enzymes such as proteases and hyaluonidase and other contents of the acrosome of the sperm are released.

- Liberation of these enzymes helps the sperm to pass through the zona pellucida and reach the plasma membrane of the oocyte.
- These interactions enable the sperm to enter and thus fertilize the oocyte.
- It may be possible to inhibit fertilization by developing drugs or antibodies that interfere with the normal functions of ZP3 and thus they act as contraceptive agents.

ROLE OF GLYCOPROTEIN–SELECTIN IN INFLAMMATION[17]

- Leukocytes play important roles in many inflammatory and immunologic phenomena.
- Leukocytes and endothelial cells contain on their surfaces specific lectins, selectins that participate in their intercellular adhesion.
- Selectins are single – chain Ca^{2+} binding transmembrane proteins that contain a number of domains.
- Their aminoterminal ends contain the lectin domin, which is involved in binding to specific carbohydrate ligands.
- The adhesion of neutrophils to endothelial cell of post capillary venules occur in four stages.

Stage I - Slowing or rolling of the neutrophils mediated by selectins.

Stage II - Interactions between L-Selectin on the neutrophil surface and CD34 and GlyCAM -1 on the endothelial surface.

Stage III - Activation of the neutrophils by various chemical mediators resulting in a change of shape of the neutrophils and firm adhesion of these cells to the endothelium.

- LFA-1 (Lymphocyte function – associated antigen–1) and Mac -1 (Integrins) on the neutrophils and ICAM -1 and ICAM -2 (Inter cellular adhesion molecule) are involved in firm adhesion.

Stage IV - Transmigration of the neutrophils across the endothelial wall. Platelet – endothelial cell adhesion molecule – 1 (PECAM -1) localized at the junction of endothelial cells and plays a role in transmigration.

The three selectin molecules P – selectin, L-selectin, E-selectin bind sialylated and fucosylated oligosaccharides and in particular all

three bind sialyl Lewis ^{x}, a structure present on both glycoprotein and glycolipids.

MUCIN– GLYCOPROTEIN HAVE HIGH CONTENT OF 0-LINKED OLIGOSACCHARIDES:-

Mucins are glycoproteins having two major characteristics:-

1) High content of O-linked oligosaccharides. The carbohydrate content of mucin in more than 50%.
2) Presence of repeating Amino acid sequence (tandem repeats) in the centre of their polypeptide backbones, to which the O-glycan chains are attached in clusters. These sequence are rich in serine, threonine and proline.

TYPES OF MUCINS

1) Secretory Mucin
2) Membrane bound Mucin

- The high content of O-glycans confers an extended structure on mucins.
- The extended structure contributes to their high viscoelasticity.
- This is due to the steric interactions between their Gal Nac moieties and adjacent amino acids, resulting in a chain – stiffening effect.
- The high content of NeuAc and sulfate residue in mucins confers a negative charge on them.

BIOSYNTHESIS OF GLYCOPROTEINS

Liver is a major source of the serum glycoproteins. This is based on the observation by Werner that the serum Glycoprotein content of rabbit serum was increased following removal of blood from rabbits. This increase is no longer occurred when liver damage was produced by intoxication with phosphorus or Benzene. One this basis, Werner.I[20] suggested that the glycoproteins may play a role in the synthesis of serum proteins in the liver.

The possibility that the liver is involved in the synthesis of some of the components of seromucoid is suggested by the observations of Greenspan et al.

SYNTHESIS OF O-LINKED GLYCOPROTEINS:-

- The polypeptide chains of O-linked and other glycoprotein are encoded by mRNA species.
- These Glycoproteins are built up by the stepwise donation of sugars from nucleotide sugars such as UDP – GalNac, UDP – Gal and CMP-NeuAc.
- The enzymes catalyzing this type of reaction are membrane bound glycoprotein glycosyl transferases.
- The factors that determine which specific serine and threonie residues are glycosylated have not been identified but are probably found in peptide structure surrounding the glycosylation site.

- The enzymes assembling O-linked chains are located in the Golgi apparatus, sequentially arranged in an assembly line with terminal reactions occurring in the trans – Golgi compartments.

SYNTHESIS OF N-LINKED GLYCOPROTEIN[21]

- N-linked glycoprotein acquires their initial sugar from Dolichol donors.
- The oligosaccharide destined for attachment to the asparagine residue of a protein is assembled attached to dolichol phosphate, a specialized lipid molecule contaning as many as 20 isoprene (C_5) units.
- Dolichol phosphate resides in the Endoplasmic Reticulum membrane
- The terminal phosphate group is the site of attachment of the activated oligosaccharide and is transferred to the protein acceptor.

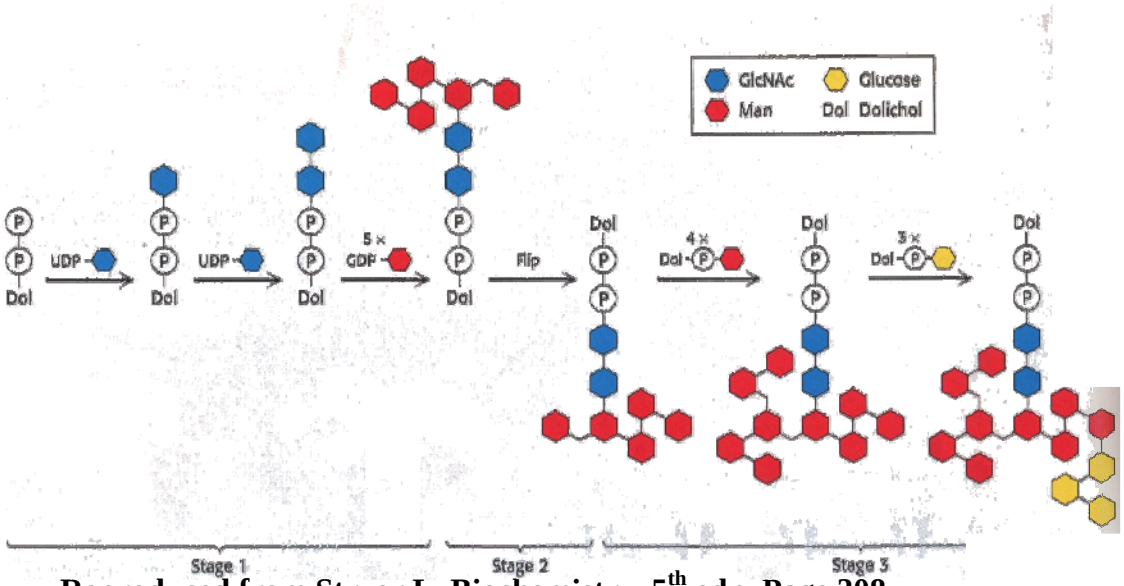
STAGES OF ASSEMBLY:-

1) 2 N-acetylglucosamine residues and 5 mannose residues are added to the dolichol phosphate through the action of a number of cytoplasmic enzymes that catalyse monosaccharide transfer from sugar nucleotides.
2) This structure is then “Flipped” through the ER membrane into the lumen of ER.

3) Finally, additional enzymes are added by enzymes in the ER lumen. This result in the formation of a 14 residue oligosaccharide attached to dolichol phosphate.

4) This is then transferred en block to a specific asparagine residue of the growing polypeptide chain.

Assembly of an N-linked Oligosaccharide precursor on Dolichol phosphate.



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PROTEIN GLYCOSYLATION[21]

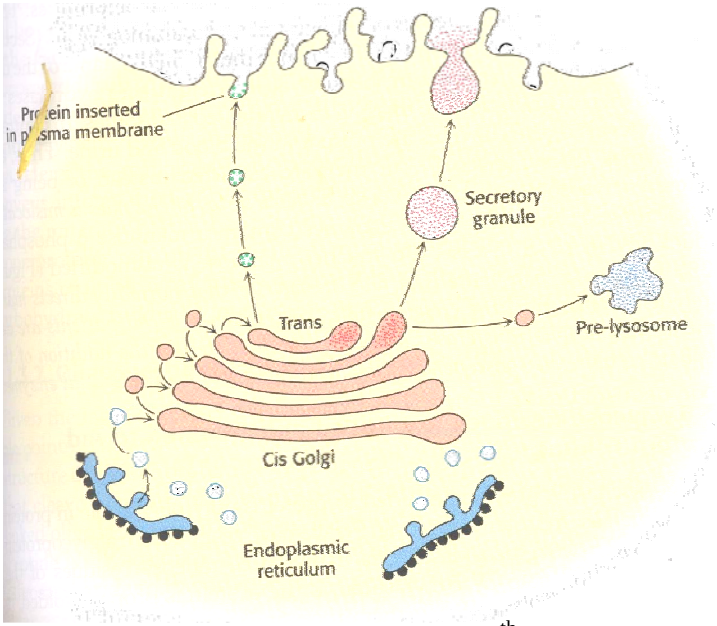
- Protein glycosylation takes place inside the lumen of the Endoplasmic reticulum and golgi complex.
- The N-linked glycosylation begins in the ER and continues in the Golgi complex.

- The O-linked glycosylation takes place exclusively in the golgi complex.

TRANSPORT OF THE PROTEIN FROM ER TO GOLGI FOR FURTHER GLYCOSYLATION AND SORTING[21]

- Proteins in the lumen of ER and in the ER membrane are transported to the golgi complex.
- The carbohydrate units of glycoproteins are altered and elaborated in the golgi complex.
- The proteins proceed from golgi complex to lysosomes, secretory granules according to the signals encoded within their Amino acid sequence and three – dimensional structures.

GOLGI COMPLEX AS SORTING CENTRE



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PHYSIOLOGICAL SIGNIFICANCE OF SERUM GLYCOPROTEINS[2]

Sibert F.B. et al suggested the elevations of Glycoprotein above normal level reflect process of tissue destruction. In support of the view that tissue destruction contributes directly to the serum glycoprotein levels is the observation that more glycoprotein carbohydrate is found in venous blood than in arterial blood.

Direct release of preformed glycoprotein from inflamed tissue is a possibility. Equally possible is the increased local synthesis and liberation of the glycoprotein by inflammation tissue.

The increased serum glycoprotein levels in disease reflect in whole or in part, processes associated with tissue proliferation[26,27]. Elevations in serum glycoproteins are frequently associated with conditions in which destruction is not pronounced and cell proliferation or protein syntheis is rapid. Eg. prostatic hyperplasia, pregnancy.

Elevated serum glycoproteins may represent a systemic response to non-specific stress. The wide variety of conditions which result in high serum glycoprotein levels would tend to support this contention and would suggest that the levels of serum glycoproteins might be affected by the pituitary adrenal axis.

DEPRESSIVE DISORDER:

Major Mood disorders comprised of Mood episodes including Major depressive episode, Manic episode, Mixed episode and hypomanic episode.

DEFINITION[22]

The term depression can define an affect, mood state, a disorder or syndrome, or a specific entity.

A depressed 'affect' usually occurs in response to a specific situation and is best defined as a relatively transient state of feeling 'depressed', 'sad' or 'blue'.

A depressed 'Mood' is more pervasive, experienced as unusual or atypical, associated with negative ideas and may influence behaviour.

A depressed 'condition' is distinguished by a longer duration, by more or greater number of clinical features and by distinct social impairment. The addition of several clinical features informs about severity i.e major and minor depressive disorders.

Depressive conditions are described as primary or secondary.

'Secondary depression' emerges during the course a substantive psychiatric condition eg. Schizophrenia or Medical condition or following triggering factors like organic states, substance abuses including alcoholism.

HISTORY[23] :-

Mood disorders were recognized in ancient times. In the late 1800s and early 1900s Emil Kraeplin distinguished the syndromes of depression and mania from the deteriorating course of schizophrenic illness in adult population.

In psychiatric diseases, Major depressive disorder is most common and it may range from a very mild condition, bordering to normality, to severe depression accompanied by hallucinations and delusion.

EPIDEMIOLOGY: - PREVALENCE

The life time prevalence of Major depressive disorder in adolescents is between 15-20% similar to that in adult population.

- 40-70% of children and adolescents have comorbid psychiatric disorders and 50% have two or more comorbid disorder.
- 10-80% of the time disruptive behaviour disorders co-occur with major depressive disorder.

MAJOR DEPRESSION	PREVALENCE RATE (Point or 1 – year)
Pre schoolers	0.3%
Children	0.4-3%
Adolescents	0.4-6.4%

- Race, Poor school performance and socio economic status were inconsistently associated with depressive disorders among youths

ETIOLOGY OF DEPRESSIVE DISORDER[23]

1. GENETICS:-

Genetic factors are of major importance as risk factors for vulnerability to major depression.

Traditional estimates have put the heritability at about 40%.

- Kindler et al estimated heritability by about 70%.
- The genes for Major depression do not appear to be unique for depression, but overlap with the genes for anxiety and the genes for neuroticism
- Wilhelm et al, suggested that the greater prevalence of depression in women is due to the strong association of anxiety and neuroticism with depression.

- The higher rates of anxiety and neuroticism in women lead to higher rates of depression.

2. GENDER:-

- The ratio of women to men in Major depression is approximately 2:1
- The increased rate of Major depression in women arises during puberty.
- In childhood, there is a slightly higher prevalence of depression in boys than girls.

3. COGNITIVE FACTORS:-

- Cognitive distortions and negative attributions are commonly observed among depressed children, adolescents and adults.
- The notion of learned helpless, first proposed by Martin Seligman describes the cognitive experience of a person who perceives unpredictable and uncontrollable events as the basis for feeling helpless and powerless.
- Another form of cognitive distortion observed in depressed persons is the belief that they are responsible for negative events that may be uncontrollable.

4) ENVIRONMENTAL FACTORS:-

- Marital status is a risk factor for Major depression.
- Separated or divorced men have the highest rates of Major depression than married men.
- Lack of paid employment is also a risk for the development of an episode of depression.
- Family conflict is the more frequent precipitating event in adolescents with suicidal behaviour.

5) ADVERSE LIFE EVENTS

- 70% of children and adolescents with new-onset depression have an adverse life event in the 12 months preceding the depression compared with 29% controls.
- Stressful life events include interpersonal losses, relationship failures divorce, bereavement and exposure to a suicide.
- A study of Major depression in preschoolers found that 100% of the subjects had been victims of neglect or abuse.

6) CHILDHOOD EXPERIENCES:-

- Loss of a parent in Child hood increased the later risk for Major depression.
- Lack of parental care is associated with increased rates of depression.

- Child hood sexual abuse is established as a risk factor for adult Major depression.

7) PERSONALITY:-

- Individuals who are unduly anxious, impulsive and obessional may have increased rate of later major depression.

8) PHYSICAL ILLNESS:-

- A chronic or severe physical illness is associated with an increased risk for depression.
- In Parkinson's disease, there are shared neurotransmitter abnormalities between the disease and depression.
- In Post – stroke depression, there is good evidence that the location of the lesion contributes to the rate of depression.
- This suggests a neurotansmiter connection between the physical illness and likelihood of depression.

9) BIOLOGICAL FACTORS:-

1) Hypothalamic – pituitary axis

About half of all patients with Major depression have a raised cortisol output, which tends to return to normal on recovery. Cortisol is always regarded as a stress hormone and is severed in response to various types of acute stress. The idea that there is a relatively specific

link between chronic high cortisol level and mood disorder is notably persistent.

It has also been suggested that elevated serum glycoproteins may represent a systemic response to non-specific stress[2]. The wide variety of conditions which result in high serum glycoprotein levels tend to support this contention and would suggest that the levels of serum glycoprotein might be affected by the pituitary – adrenal axis.

Abnormal cortisol secretion in adults with major depressive disorder was demonstrated with overall elevation of 24hr basal secretion, non suppression on a Dexamethasone challenge test, (DST) blunting of normal diurnal rhythms and increased nocturnal cortisol secretion.

2. SEROTONIN:-

Some evidence in adult studies indicates that a dysregulation of the serotonin system might contribute to the development of depression. This hypothesis is based on the blunted response of cortisol secretion to serotonergic agents used to challenge the system.

Other studies have found that brains of suicide completers contain an increased postsynaptic Number of serotonin receptors in the prefrontal cortex, suggesting that this increase in receptors may be a mechanism to compensate for decreased serotonin release.

3. GROWTH HORMONE : -

Growth hormone secretion i.e blunted in response to insulin – induced hypoglycemia during the depressive episode as well as after resolution of depression.

Blunted growth hormone response may be a trait marker for depression since it occurs among children who have had depression even after the depression has remitted.

CRITERIA FOR DIAGNOSING DEPRESSIVE DISORDER

- There are number of criterias for depression which includes Diagnostic and statistical Manual of Mental disorders III and IV (DSM III & IV) and the 10^{th} revision of International statistical classification of Diseases and Related Health problems (ICD – 10), according to WHO. (World Health organization)
- The present study on Depressive disorder patients is based on ICD – 10 criteria.

ACUTE DEPRESSION AS DEFINED IN DSM – IV AND ICD – 10

	Symptoms of Depression	DSM – IV	ICD - 10
1.	Depressed Mood most of the day, nearly every day	+	+
2.	Markedly diminished interest or pleasure in all or almost all, activities most of the day, nearly every day	+	+
3.	Loss of energy or fatigue nearly every day	+	+
4.	Loss of confidence or self – esteem	-	+
5.	Unreasonable feeling of self – reproach or excessive or inappropriate guilt nearly every day	+	+

6.	Recurrent thoughts of death or suicide or suicidal behaviour	+	+
7.	Diminished ability to think or concentrate or indecisiveness, nearly every day	+	+
8.	Psychomotor agitation or retardation nearly every day	+	+
9.	Insomnia or hypersomnia nearly every day	+	+
10.	Change in appetite (decrease or increase) with corresponding weight change	+	+

CLASSIFICATION OF AFFECTIVE MOOD DISORDERS

a) Formal classification

b) Hierarchical or tiered model – clinical classification.

FORMAL CLASSIFICATION:-

The ICD – 10 systems classify the depressive disorder into 3 types.

1) Mild depressive episode

2) Moderate depressive episode

3) Severe depressive episode.

In the present study, the patients with Depressive disorder are diagnosed based on ICD – 10 criteria.

GENERAL CRITERIA (F32)

1) Depressive episode should last for at least 2 weeks
2) No hypomanic or manic symptoms at anytime in the individual's life.
3) Episode not attributable to psychoactive substance use or to any organic mental disorder.

MILD DEPRESSIVE EPISODE (F32.0)

A) General criteria must be met.

B) At least 2 of 3 following symptoms:-

a) Depressed Mood to a degree which is abnormal for the individual present for most of the day and almost every day. Uninfluenced by circumstances and sustained for at least 2 weeks.

b) Loss of interest or pleasure in activities that are normally pleasurable.

c) Decreased energy or increased fatigability

C) ANY 4 OF THE FOLLOWING SYMPTOMS:-

a) Loss of confidence or self – esteem

b) Unreasonable feeling of self reproach or excessive and inappropriate guilt.

c) Recurrent thoughts of death or suicide or any suicidal behaviour.

d) Complaints or evidence of diminished ability to think or concentrate such as indecisiveness or vacillation.

e) Change in psychomotor activity with agitation or retardation.

f) Sleep disturbance of any type.

g) Change in appetite decreased or increased with corresponding weight change.

MODERATE DEPRESSIVE EPISODE (F32.1)

A) General Criteria must be met.

B) At least 2 or 3 symptoms from F32.0 Criteria B.

C) Additional symptoms from F32.0 Criteria C, to give a total of at least Six.

SEVERE DEPRESSIVE EPISODE (F32.2)

A) General Criteria F32

B) All 3 symptoms in criteria B, F32.0

C) Additional symptoms from F32.0 Criteria C to give a total of at least 8.

D) No hallucinations, delusion or depressive stupor.

MATERIALS AND METHODS

The present study was carried out after getting approval from the Ethical Committee, Stanley Medical College, Chennai.

The study was carried out for a period of four months from June, 06 to September 06.

The patient with Depressive disorder was selected from the outpatient department, Department of psychiatry, Stanley Medical College Hospital, Chennai and Madras Medical College Hospital, Chennai.

The blood samples were analyzed and serum glycoprotein levels were estimated as protein bound hexose and protein bound hexosamine in the Department of Biochemistry Stanley Medical College, Chennai.

The study was carried out on 100 subjects comprising 50 normal healthy volunteers and 50 patients with severe depressive disorder with the range of age 25-55 yrs.

Healthy Normal subjects were selected on the basis of good health as evidenced by the Medical history, complete physical examination and routine laboratory tests performed prior to the commencement of the study. Informed consent was obtained from the healthy normal subjects.

CASE SELECTION:

The diagnosis of patients with Severe Depressive disorder was performed using International classification of Disease – 10 criteria (ICD – 10) by a clinical psychologist. Informed and written consent was obtained from the legal guardian of the patients.

ICD – 10 criteria for the diagnosis of Depressive disorder[24].

General criteria (F32)

1) Depressive episode should last for at least 2 weeks.
2) No hypomanic or Manic symptoms at any time in the individual's life.
3) Episode not attributable to psychoactive substance use or to any organic mental disorder.

MILD DEPRESSIVE EPISODE (F 32.0)

A) General Criteria(F 32) must be met.

B) At least 2 – 3 following symptoms : -

b) Depressed mood to a degree which is abnormal for the individual present for most of the day and almost every day uninfluenced by circumstances and sustained for at least 2 weeks.

c) Loss of interest or pleasure in activities that are normally pleasurable.

d) Decreased energy or increased fatigability.

C) Any 4 of the following symptoms :-

a) Loss of confidence or self – esteem

b) Unreasonable feeling of self reproach or excessive and inappropriate guilt.

c) Recurrent thoughts of death or suicide or any suicidal behaviour.

d) Complaints or evidence of diminished ability to think or concentrate such as in decisiveness or vacillation.

e) Change in psychomotor activity with agitation or retardation.

f) Sleep disturbance of any type

g) Change in appetite increased or decreased with corresponding weight change.

MODERATE DEPRESSIVE EPISODE : - (F32.1)

A) General Criteria F32 must be met.

B) At least 2 of 3 symptoms for F 32.0 criteria B must be present.

C) Additional symptoms from F32.0 criteria C, to give a total of atleast 6.

SEVERE DEPRESSIVE EPISODE :- (F32.2)

A) General Criteria F32 must be met.

B) All three symptoms in criteria B, F32.0 must be present.

C) Additional symptoms from F32.0 Criteria C to give a total of at least 8.

D) No Hallucinations, delusion or depressive stupor.

EXCLUSION CRITERIA

- H/o. Cardiovascular disease
- H/o. Pulmonary tuberculosis
- H/o. Trauma, prolonged bed rest
- H/o. carcinoma cervix, breast
- H/o. chronic alcoholism
- H/o. diabetes, hypertension

SAMPLE COLLECTION AND PROCESSING:-

Blood samples (3-5ml) were collected from the normal subjects and from the patients with severe depressive disorder. After collection the blood samples were centrifuged to separate the serum. The Biochemical analysis was performed on serum samples for estimation of protein bound hexose and protein bound hexosamine. All the reagents were of analytical reagent (AR) grade.

ESTIMATION OF SERUM GLYCOPROTEINS[2]:-

1. Protein Bound hexose :-

Protein bound hexose was estimated by the method of Weimer.H.E., and Moshin.J.R.

In this Method, the hexose moiety of glycoprotein conjugates precipitated by ethanol at room temperature. Is then determined by orcinol reaction and read at 540nm.

STANDARDIZATION OF THE PROCEDURE: -

A galactose – Mannose standard is employed as the serum glycoproteins contains only these two hexoses in approximately equal amounts. Since the optical density varies with the different hexoses, the standard should contain the same sugars in the same proportions as occur in the unknown.

Hexosamine and fucose components known to be present in serum glycoproteins, do not interfere with the determination at 540nm

Preparation of standard solution:-

Galactose – Mannose standard 0.2mg/ml = 0.1mg/ml each of galactose and Mannose.

- Stock solution – 100mg each to galactose and Mannose was dissolved in 100ml of deionised water.

- Working standards of different conversation prepared from the stock solution.

Working standard concertration	Stock solution (ml)	Deionised water (ml)
10mg%	1	9
20mg%	2	8
30mg%	3	7
40mg%	4	6
50mg%	5	5

REAGENTS

1) 95% Ethanol

2) Orcinol – H_2S04 Reagent 7.5 volumes of Reagent A mixed fresh daily with 1 volume of Reagent B.

   Reagent A : 60ml con. H_2S04 and 40ml of H_2^0

   Reagent B : 1.6gml of orcinol in 100ml H_20

   0.2mg/ml Galactoso – Mannose standard.

PROCEDURE:

1) To 0.1ml of serum in a 15x150mm test tube, 5ml of 95% ethanol was added and mixed.

2) Centrifuged for 15 minutes, decanted and suspended the precipitate in 5ml of 95% ethanol centrifuged and decanted.

3) Dissolved the precipitated proteins in 1ml of 0.1N NaoH.

4) Blank – 1ml of H_20 and 1ml of standard solution was taken in a test tube.

5) 8.5 ml of orcinol – sulphuric acid Reagent is added to all the tubes and mixed well by inversion.

6) The tubes ware capped with glass marbles to minimize evaporation and placed in a water bath at 80^0C for 15 minutes.

7) The tubes were then cooled in tap water and the readings were observed in spectrophotometer at 540nm.

CALCULATION:-

$$\text{Concentration of Protein bound hexose (mg\%)} = \frac{\text{Test OD}}{\text{Standard OD}} \times \frac{\text{Concentration of standard}}{\text{Test volume}} \times 100$$

2) PROTEIN BOUND HEXOSAMINE:-

Protein bound hexosamine was estimated by the method of Winzler.R.J

The Serum Proteins are precipitated by ethanol. The hexosamine is liberated from the glycoproteins by acid hydrolysis. It is acetylated with acetyl acetone and is treated with alkali to form a cyclic oxazole or pyrrole, and coupling with P-dimethylaminobenzaldehyde (Ehrlich's

Reagent) to form a coloured derivation which is read photometrically at 530nm.

STANDARDIZATION:-

The standard used was glucosamine. The optical densities given by galactosamine are the same as those given by glucosamine, so that the use of glucosamine standard is justified.

PREPARATION OF STANDARD SOLUTION:

Stock solution was prepared by dissolving 100mg of Glucosamine hydrochloride in 100ml of deionised water.

Working standard was prepared form 100mg% stock solution.

Working standard concentration	**Stock solution (ml)**	**Deionised water (ml)**
6mg%	0.6	9.4
9mg%	0.9	9.1
12mg%	1.2	8.8
15mg%	1.5	8.5
18mg%	1.8	8.2

REAGENTS :-

1) 95% Ethanol

2) 3N Hcl

3) 3N NaoH

4) Acetyl acetone Reagent – 1ml of acetyl acetone in 50ml of 0.5N Na_2 co_3 freshly prepared.

3) Ehrlich's Reagent – 0.8g of P-dimethylaminobenzaldehyde dissolved in 30ml of methanol and 30ml of conc. Hcl

4) Glucosamine standard 0.06mg glucosamine Hcl/ml.

PROCEDURE :-

1) To 0.1ml of serum in a 15 x 150 mm test tube, 5ml of 95% ethanol is added and mixed.

2) Centrifuged for 15 minutes, decanted and the precipitate was resuspended in 5ml of 95% ethanol, centrifuged and decanted.

3) To the precipitated proteins, 2ml of 3N Hcl is added and is hydrolyzed in a boiling water bath with air condenser for 4hrs.

4) The hydrolysate was neutralized with 3N until it is barely alkaline to litmus and diluted to 10 ml.

5) To 1ml aliquots and to 1ml of water for a blank and 1ml of glucosamine standard 1ml of acetylacetone reagent is added and mixed.

6) The tubes were capped with marbles to prevent evaporation and placed in the boiling water bath for 15min.

7) The takes were cooled in tap water, and 5ml of 95% ethanol is added and mixed.

8) To this mixture, 1ml of Ehrlich's reagent is added, mixed well and diluted to 10ml with 95% Ethanol.

9) Readings was observed in spectrophotometer after 30 min at 530nm.

Calculation :

$$\text{Concentration of protein bound hexosamine (mg\%)} = \frac{\text{Test OD}}{\text{Standard OD}} \times \frac{\text{Test volume}}{\text{Concentration of Standard.}} \times 100$$

RESULT AND STATISTICAL ANALYSIS

- Mean and standard deviation were estimated for each study group i.e cases and controls.
- Data were expressed as mean ± S.D
- Data so obtained was analyzed to obtain appropriate conclusions.
- Mean values were compared between cases and controls by using students independent‘t’ test.
- Student‘t’ test was employed to find out the ‘p’ value.
- Pearson’s correlation analysis was done to assures the relationship between the parameters, protein bound hexose and protein bound hexosamine in each study group.
- The results of the present study are presented in Table 1.

TABLE 1

Mean standard deviation and test of significance of mean values between cases and controls.

VARIABLE	CASE	CONTROL	P.VALUE
	Mean ± S.D (mg%)	**Mean ± SD (mg%)**	
Protein bound hexose	191.05 ± 4.01	102.50 ± 5.81	< 0.0001
Protein bound hexosamine	97.50 ± 5.06	78.50 ± 4.97	< 0.0001

INFERENCE – TABLE 1

- Mean protein bound hexose level in cases (191.05±4.01) was significantly higher than control (102.50 ± 5.81). P < 0.0001.
- Mean protein bound hexosamine in cases (97.50±5.06) was significantly higher than control (78.50 ± 4.97). P < 0.0001.

Correlation between P_1 & P_2 in each study group

TABLE 2

Group	Correlation co efficient (r)	P-Value
Control	0.69	<0.0001
Cases	0.42	0.047

INFERENCE (TABLE 2)

For controls, there was a significant positive correlation between protein bound hexose and protein bound hexosamine (r=0.69, P<0.0001)

For cases, there was a positive correlation between PBH, PBHx and is statistically significant r=0.42 P=0.047.

In the present study, P<0.05 was considered as the level of significance.

DISCUSSION

In this study, a significant difference in mean concentrations of protein bound hexose and protein bound hexosamine was observed between normal healthy subjects and patients with severe depression.

This study demonstrates that the level of serum glycoproteins protein bound hexose and protein bound hexosamine were elevated in Severe depressed patients relative to the control group.

Narendra G. Mehta and Alamela Ventakaraman[10] had reported that the concentration of the serum glycoprotein is elevated in patients suffering from wide variety of pathological conditions.

Elevated serum glycoproteins represent a systemic response to non-specific stress and levels are affected by the pituitary adrenal axis.

Depression is associated with high cortisol level and hyperactivity of hypothalamic pituitary adrenal axis[25,26]. This probably explains the raised level of protein bound hexose and protein bound hexosamine in patients with depression.

Depression also exhibits certain immune disturbance during acute episode as evidenced by high levels of serum alpha – 1- acid glycoprotein, increased C – reactive protein[12] . Serum alpha – 1- acid

glycoprotein raise as a result of increased hepatic synthesis stimulated by inflammatory cytokines[27].

Thus it is suggested from the present study that there is increase in serum levels of protein bound hexose and protein bound hexosamine in patients with severe depression. This finding is due to glycosylation of proteins or be due to increased hepatic biosynthesis of glycoproteins or due to release of preformed proteins in patients of depression.

CONCLUSION

The present study shows the diagnostic relevance of serum glycoproteins in depressive disorders. Although serum glycoprotein levels appear to be one of the non-specific indicators of depression, it can be concluded on the basis of present study that serum glycoprotein levels serve as a useful marker in the diagnosis of patients with depressive disorders.

MASTER CHART

CASES

Sl.No.	Name	Age	PB Hexose (Conc)mg%	PB Hexosamine (Conc)mg%
1)	Mallik Basha	53	194.47	100.00
2)	Srinivasan	49	185.08	90.00
3)	Ramesh	31	205.00	101.00
4)	Mohandass	32	183.42	88.99
5)	Jayanthi	27	187.84	88.00
6)	Jessy	25	181.21	110.00
7)	Panchavarnam	34	193.37	97.99
8)	Jamuna	28	192.81	93.00
9)	Mehabob	30	185.08	99.00
10)	Kavali	54	192.26	91.99
11)	Muthusamy	38	191.71	88.00
12)	Usha	42	187.29	88.99
13)	Stalin	29	192.26	110.00
14)	Thangammal	36	187.29	94.99
15)	Sridhar	43	187.84	99.00
16)	Veni	44	191.16	100.00
17)	Jamuna	35	186.74	101.00
18)	Mariappan	45	195.02	90.00
19)	Munisamy	33	191.16	101.00
20)	Karuppaiah	51	190.60	99.00
21)	Muthulakshmi	38	193.92	97.99
22)	Prema	36	188.95	94.99
23)	Arumugam	45	191.71	91.00
24)	Govindasamy	41	190.60	94.00
25)	Hari	27	191.16	99.00
26)	Krishnapriya	29	191.71	97.99
27)	Mani Megalai	31	194.47	100.00

28)	Dhanabakiyam	33	195.02	110.00
29)	Nazeer Ahmed	42	193.92	101.00
30)	John	46	194.47	99.00
31)	Mallika	52	191.16	97.99
32)	Ilayaraja	32	191.71	91.99
33)	Kumar	28	187.84	94.00
34)	Maheswari	31	192.26	100.00
35)	Jamuna	36	187.29	94.99
36)	Premkumar	39	195.02	100.00
37)	Arul	40	188.95	97.99
38)	Sabeena Banu	28	185.08	94.99
39)	Radhika	27	193.37	101.00
40)	Josephin	25	194.47	97.99
41)	Nafeeza	39	193.92	97.00
42)	Munirathinam	34	191.71	97.00
43)	Lalitha	29	195.02	101.00
44)	Ezhil	30	193.37	97.99
45)	Suresh	26	194.47	100.00
46)	Govindasamy	49	192.26	99.00
47)	Munirathinam	51	193.37	101.00
48)	Rasathi	42	185.08	97.99
49)	Padmavathi	32	191.16	99.00
50)	Meenakshi	36	187.84	91.99

CONTROLS

Sl.No.	Name	Age	PB Hexose (Conc)mg%	PB Hexosamine (Conc)mg%
1.	Mani	31	111.10	80.00
2.	Veni	27	106.07	70.00
3.	Ramkumar	29	105.00	82.00
4.	Gayathri	34	98.89	85.00
5.	Ganesh	40	105.00	80.00
6.	Madhavi	26	116.02	85.00
7.	Karunanidhi	52	111.10	84.00
8.	Saroja	41	99.44	79.00
9.	Annie	28	100.55	80.00
10.	Savithri	33	105.00	82.00
11.	Chellammal	38	94.60	79.00
12.	Meenakshi	37	100.55	81.00
13.	Subitha	30	111.10	83.00
14.	Kousalya	44	105.52	80.00
15.	Rajan	48	94.60	75.00
16.	Mohan	39	105.50	82.00
17.	Kanimozhi	29	88.39	60.00
18.	Arumugam	41	94.60	70.00
19.	Jayanthi	35	100.55	80.00
20.	Sengamalam	35	106.07	82.00
21.	Chandran	32	109.18	84.00
22.	Kannan	49	102.20	79.00
23.	Srinivasan	46	106.01	82.00
24.	Jamuna	39	94.06	70.00
25.	Anitha	36.	105.52	80.00

26.	Dinesh	29	88.80	70.00
27.	Kamalam	40	105.50	81.00
28.	Radha	37	106.07	82.00
29.	Chelladurai	38	100.55	71.00
30.	Ramakrishnan	46	98.34	71.00
31.	Jeyaraman	27	100.00	70.00
32.	Latha	29	98.89	71.00
33.	Devi	35	106.07	80.00
34.	Maheswari	52	108.28	81.00
35.	Lakshmi Priya	32	97.79	79.00
36.	Sivashanmugam	29	107.18	81.00
37.	Ramesh	32	102.20	80.00
38.	Krishnan	45	100.55	79.00
39.	Karthika	26	105.52	81.00
40.	Selvavinayagam	45	92.26	78.00
41.	Vijayalakshmi	42	108.28	82.00
42.	Sakthi	30	106.62	81.00
43.	Venu	28	106.07	81.00
44.	Thangam	32	102.20	79.00
45.	Thambidurai	29	94.60	78.00
46.	Mayilu	39	105.00	81.00
47.	Kandhasamy	40	106.07	80.00
48.	Thangavelu	38	98.89	78.00
49.	Lakshmi Narayanan	25	102.20	79.00
50.	Perumal	35	99.44	77.00

PROFORMA

Date :

Stanley Medical College Hospital

Name : OP No..

Age :

Sex :

Occupation :

Clinical History :

On Examination :

Investigation :

1) Protein Bound Hexose : mg/dL

2) Protein Bound Hexosamine: mg/dL

BIBLIOGRAPHY

1) Winzler R.J. Advances in Cancer Research Vo.1. Academic Press, Newyork , 1953. p 503.
2) Winzler R.J. (1955). Determination of serum glycoproteins. Methods of biochemical analysis. Vo.2. Ed. Glick page 299-311, Interscience Publishers, Newyork, USA.
3) U. Singh, R.L. Solanki, V.K. Anand and I.N. Ramdeo (1989) A Study of Serum Glycoprotein in Pulmonary tuberculosis.
4) Dutt, M. Chatterjee, A.K. Khanade J.M. and Rajan S.R. (1975). Serum Glycoproteins in patients of Ischemic Heart Disease and Normal Controls. Indian J. Med. Res. 63, 282 – 285.
5) Bhuvarahamurthy, V. Balasubramanian, N. Vijayakumar S. and Govindswamy S (1995) : Effect of radiation on serum glycoproteins and glycosidases in patients with cervical carcinoma. Int. J. Gynaecol. Obstet, 48, 49-54.
6) Varma, R. and Hoshino. A.Y. (1980) Serum glycoprotein in Schizophrenia. Carbohydr. Res. 82, 343-351.
7) Mukesh Nandav, S.K. Ojha, Ranjit Kaur. Changes in levels of serum glycoproteins in major depressive disorders. Indian Journal of clinical biochemistry, 2005, 20(2) 154-157.
8) Opeolu M. Adeoye, Robert E. Ferrell, Margaret A.Krishner, Benoit H. Mulsant, Karen Seligman, Amy E. Begley, Charles F. Reynolds III, and Bruce G. Pollock. Journal of Geriatric Psychiatry and Neurology. Vol.16, No.4 Dec. 2003.
9) Nieto E, Vieta E, Alvaroz L, et al. Alpha 1 – acid glycoprotein in Major depressive disorder. Relationship to severity, response to treatment and imipramine plasma levels. J. affect disord 2000; 59 : 159 – 164.
10) Narendra G. Mehta and Alamela Ventakaraman (1973). Serum Glycoproteins levels and disease – an evaluation. Indian J Med. Res. 63, 970 – 976.
11) Fatemi SH, Kroll JL, Stary J.M. Altered levels of Reelin and its isoforms in mood disorders and schizophrenia. Neuroreport 2001 Oct. 29; 12(15) : 3209 – 15.
12) Sluzewska A, Rybakowski JK, Sobieska M, Wiktorowicz K. Concentration and Microheterogenicity glycophorms of alpha – 1- acid glycoprotein in Major depressive disorder. J. Affect Disord. 1996 Jul. 8 ; 39(2) ; 149-55.
13) Bondy B, Baghai TC, Minov C, Schule C, Schwarz MJ et al. Substance P serum levels are inceased in Major depression. Biol Psychiatry, 2003 Mar. 15 ; 53 (6) : 538 – 42.
14) Healy D, Calvin J, White house AM et al. Alpha-1- acid glycoprotein in major depressive and eating disorders. J. Affect disord. 1991; 22 : 13-20.

15) Kehoe WA, Kwentus JA, Sheffel WB, Harralson AF, Increased alpha-1-acid glycoprotein in depression lowers free fraction of imipramine. Biol Psychiatry 1991; 29: 489 – 493.
16) Bruce EL, Mussel Man DL. Depression, alterations in platelet function and Ischemic heart disease. Psychosom Med. 2005; 67 (Suppl 1) : 534 – 6.
17) Robert K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rod Well. Harper's Illustrated Biochemistry. 26th Edition Page 514 – 534. Mc. Graw Hill.
18) Schmid K.J. Am. Chem. Soc. 75, 2532 (1953).
19) Robert K. Murrey, Daryl K. Granner, Peter A : Mayer Victor W. Rodwell. Harper's Biochemistry 25th Edition Page 675 – 694 Mc. Graw Hill.
20) Werner, I. Acta Physiol. Scand 19, 27 (1949).
21) Jeremy M. Berg, John L. Tymoczko, Lubert Stryer, Biochemistry. 5th Edition. W.H. Feeman and company page 306 – 310.
22) M.G. Golder, Juan J. Lopoz – Ibor Jr. Nancy C. Andreasen. New oxford Text book of psychiatry. Vo.1, page 677-736. Oxford University press Inc. New york. Reprinted 2004. First published 2000.
23) Benjamin J. Sadoc, Virginia A. Sadock. Kaplan & Sadock . Comprehensive Text Book of Psychiatry. 7th Edition Vol. 2, Page 2740 – 2757. Lippincott Willians and Wilins.
24) The ICD – 10 Classification of Mental and Behavioural disorders – clinical descriptions and diagnostic guidelines. World health organization. Geneva. Page 81-87. A.I.T.B.S. Publishers and Distributors.
25) Gillespie CF, Nemoroff CB. Hypercortisolemia and depression. Psychosom Med. 2005; 67 (Suppl.1) S 26-8.
26) Shotlar M.R, Foster J.V. Koelly, K.H. Shetlar, C.L. Bryan. R.S. and Everett M.R. Cancer Research 9, 515 (1999).
27) Shetlar M.R., Kelly, K.H. Foster, J.V. shetlar, C.L. and Everdt. M.R. Am. J. Obstet. Gynecol, 59, 1140.
28) Fran Van Hunsel, Annick Waulers, Eric Vandoolaeghe et al. low total serum proteins, albumin and Beta and gamma – globulin in major and treatment – resistant depression. Effects of antidepressant treatments. Psychiatry Research Vol. 65, Issue 3, 1996 Page 159 – 169.
29) Stanley L. Robbins, Ramzi S, Cotran Kumar et al. pathologic basis of diseases. 7th Edition. Saunders Page 65.
30) Nemer off CB, Krishnan KR, Blazer DG, Knight DL, Benjamin D, Meyerson LR. Elevated plasma concentrations of α – 1 acid glycoprotein, a putative endogenous inhibitor of the tritiated imipramin binding site, in depressed patients. Arch Gen Psychiatry, 1990 Apr. 47 (4), 337-40.

31) Schuld A, Schmid DA, Haack M, Holsboer F, Friess E, Pollmacher T. Hypothalamo – pituitary – adrenal function in patients with depressive disorders is correlated with baseline cytokines levels, but not with cytokine response to hydrocortisone. J Psychiatr Res 2003, Nov – Dec 37(6); 463 – 70.
32) Seidel A, Arolt V, Hunstiger M, Renk L, Behnisch A, Kirchner H. Cytokine production and serum proteins in depression. Scand J. Immunol – 1995 Jun. 41(6) 534 – 8.
33) Maes M, Scharpe S, Cosyns P, Heltzer H. Relationships between basal hypothalamic pituitary – thyroid axis activity and plasma haptoglobin levels in depression. J Psychiatr Res, 1994 Mar – Apr 28(2); 123 – 34.
34) Song C, Dian T, Leonard BE. Changes in immunoglobulin, Complement and acute phase protein levels in the depressed patients and normal controls. J. Affect Disord 1994; Apr 30(4); 283-8.
35) Maes M, Scharpe S, Meltzer HY, Bosmans E, Suy E, Calabrese J, Cosyns P. Relationship between Interleukin – 6 activity, acutephase proteins and function of the hypothalamic – pituitary – adrenal axis in severe depression. Psychiatry Res 1993 Oct. 49(1) 11-27.
36) Joyce PR, Hawes CR, Mulder RT, Seliman JD, Wilson DA, Bosewell DR. Elevated levels of acute phase plasma proteins in major depression. Biol psychiatry 1992, Dec 1 32 (11). 1035 -41.
37) Maes M, Scharpe S, Van Grotel L, Uyttenbroeck W, Cooreman W, Cosyns P. Suy E. Higher alpha 1 antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression - further evidence for the existence of an inflammatory response during that illness. J Affect Disor 1992, Mar ; 24(3). 183-192.
38) Schlechte JA, Coffman T. Plasma free cortisol in depressive illness – a review of findings and clinical implications. Psychiatr Med. 1985; 3(1) – 23 to 31.
39) Hornig M, Goodman DB, Kamoun M, Amsterdam JD. Positive and negative acute phase proteins in affective subtypes. J affect disord 1998 Apr. 49(1); 9-18.

REVIEW OF LITERATURE

1) Fatemi SH, Kroll JL, Stary JK had studied the altered levels of Reelin and its isoforms in mood disorders (Bipolar disorder and major depression) and schizophrenia. They reported that blood Reelin levels and its isoforms may be used as potential peripheral markers to diagnose presence of several psychiatric disorders and may also serve as targets for further therapeutic interventions.
2) Sluzewska A, Rybakowski JK et al reported high level of Alpha -1-acid glycoproteins and its microheterogencity in patients with major depressive disorder.
3) Bondy B. Baghai TC, Minov C et al reported increased levels of substance 'P' in major depression.
4) Healy D, Calvin J, White house AM et al studied about the level of Alpha -1- acid glycoprotein in major depressive and eating disorders.
5) Kehoe WA, Kwentus JA, Sheffol WB, Harralson AF, reported increased alpha 1 – acid glycoprotein in depression lowers free fraction of imipramine.
6) Bruce EC, Mussel man D L studied about depression, alterations in platelet function and Ischemic heart disease. They observed that depression is also associated with complex platlet abnormalities such as increased concentrations of functional glycoproteins 11b/111a receptors.

PSYCHOLOGICAL SIGNIFICANCE OF SERUM GLYCOPROTEINS[2]

Sibert F.B. et al suggested the elevations of Glycoprotein above normal level reflect process of tissue destruction. In support of the view that tissue destruction contributes directly to the serum glycoprotein levels is the observation that more glycoprotein carbohydrate is found in venous blood than in arterial blood.

Direct release of preformed glycoprotein from inflamed tissue is a possibility. Equally possible is the increased local synthesis and liberation of the glycoprotein by inflammation tissue.

The increased serum glycoprotein levels in disease reflect in whole or in part, processes associated with tissue proliferation[26,27]. Elevations in serum glycoproteins are frequently associated with conditions in which destruction is not pronounced and cell proliferation or protein syntheis is rapid. Eg. prostatic hyperplasia, pregnancy.

Elevated serum glycoproteins may represent a systemic response to non-specific stress. The wide variety of conditions which result in high serum glycoprotein levels would tend to support this contention and would suggest that the levels of serum glycoproteins might be affected by the pituitary adrenal axis.

DISCUSSION

In this study, a significant difference in mean concentrations of protein bound hexose and protein bound hexosamine was observed between normal healthy subjects and patients with severe depression.

This study demonstrates that the level of serum glycoproteins protein bound hexose and protein bound hexosamine were elevated in depressed patients relative to the control group.

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Elevated serum glycoproteins represent a systemic response to non-specific stress and levels are affected by the pituitary adrenal axis.

Depression is associated with high cortisol level and hyperacivity of hypothalamic c pituitary adrenal axis. This probably explains the raised level of protein bound hexose and protein bound hexosamine in patints with depression.

Depression also exhibits certain immune disturbance during acute episode as evidenced by high levels of serum alpha – 1- acid glycoprotein, increased C – reactive protein and serum alpha – 1- acid glycoprotein raise as a result of increased hepatic syntheis stimulated by inflammatory cytokines.

Thus it is suggested from the present study that there is increase in serum levels of protein bound hexose and protein bound hexosamine in patients with severe depression. This finding is due to glycosylation of proteins or be due to increased

hepatic biosynthesis of glycoproteins or due to release of proformed proteins in patients of depression.

CONCLUSION

The present study shows the diagnostic relevance of serum glycoproteins in depressive disorders. Although serum glycoprotein levels appear to be one of the non-specific indicators of depression, it can be concluded on the basis of present study that serum glycoprotein levels serve as a useful marker in the diagnosis of patients with depressive disorders.